In the Claims:

Claims 1 – 51 (Canceled).

Claim 52. (NEW) A method for the measurement of biological ligand association with an insoluble surface, comprising:

[a] applying a labeled ligand, comprising a biological ligand possessing a conjugate specifically recognizable by a detectable group of agents, to a known amount of said surface, in the presence or absence of an unlabeled ligand, comprising of same said biological ligand which does not possess said conjugate, and,

[b] waiting for a period of time, and,

[c] removing the non-surface associated labeled ligand and the non-surface associated unlabeled ligand from the surface environment, and

[d] solubilizing the surface-associated labeled ligand and the surface-associated unlabeled ligand; with or without disruption of said surface, thereby producing a solubilized surface and ligand mixture, and,

[e] preparing a labeled ligand standard series, comprising a series of formulations of known amounts of the labeled ligand, and,

[f] optionally separating said solubilized surface and ligand mixture, thereby producing a separated solubilized surface and ligand mixture; while concomitantly separating, in parallel, said labeled ligand standard series, thereby producing a separated ligand standard series, and,

[g] immobilizing onto a support, 1). said solubilized surface and ligand mixture, and said labeled ligand standard series; or, 2). said separated solubilized surface and ligand mixture, and said separated ligand standard series and,

[h] detecting all the support-associated labeled ligand, by applying to said support the detectable specific conjugate recognizing group of agents, thereby producing a signal correlating with the amount of labeled ligand present on the support, and,

[i] determining the amount of the support-associated previously surface-associated said labeled ligand, by comparing its said signal to the signals obtained from the support-associated labeled ligand standard series and,

[j] using the information from [a] and [i] to determine the amount of said labeled ligand originally associated with each said surface amount.

whereby the use of radiolabeled ligand is avoided and,

whereby the sensitive detecting of said labeled ligand is afforded by the use of the conjugate recognizing agents and,

whereby the support position of said signal arising from the separated previously surface associated labeled ligand is verified by comparing it to the support position of said signals arising from said separated labeled ligand standard series and,

whereby the signals arising from previously surface associated samples containing labeled and unlabeled ligand, are compared to the signals arising from previously surface associated samples containing labeled ligand only, thereby ascertaining competition for the surface association between said labeled ligand and said unlabeled ligand, thus verifying specific labeled ligand surface association.

Claim 53. (NEW) The method of claim 52, wherein said surface includes biological cells.

Claim 54. (NEW) The method of claim 52, wherein said conjugate includes entities which are specifically recognized by an antibody.

Claim 55. (NEW) The method of claim 52, wherein said conjugate includes fluorescent labels.

Claim 56. (NEW) The method of claim 56, wherein said conjugate is selected from the group consisting essentially of, fluorescein, biotin, rhodamine, and digoxygenin.

Claim 57. (NEW) The method of claim 52, wherein said biological ligand is a biological factor, a protein, DNA, or an oligonucleotide.

Claim 58. (NEW) The method of claim 57, wherein said protein is selected from the group consisting essentially of transferrin, concanavalin A, avidin, annexin V, and insulin.

Claim 59. (NEW) The method of claim 52, wherein said separating method includes electrophoresis.

Claim60. (NEW) The method of claim 59 wherein said electrophoresis method is selected from the group consisting essentially of sodium dodecyl sulfate polyacrylamide electrophoresis, electrophoresis according to Schagger Von Jagow, and agarose electrophoresis.

. Claim 61. (NEW) The method of claim 52, wherein said immobilization method includes blotting..

Claim 62. (NEW) The method of claim 61, wherein said blotting method is selected from the group consisting essentially of dot blotting, slot blotting, or western blotting.

Claim 63. (NEW) The method of claim 52, wherein said immobilization support includes protein binding materials.

Claim 64. (NEW) The method of claim 52, wherein said immobilization support includes nucleic acid binding materials.

Claim 65. (NEW) The method of claim 52, wherein said immobilization support includes blotting membranes.

Claim 66. (NEW) The method of claim 64, wherein said blotting membrane is selected from the group consisting essentially of nitrocellulose, polyvinylidenediflouride, or nylon.

Claim 67. (NEW) The method of claim 52, wherein said detecting of said immobilization support associated labeled ligand, further comprises applying to said support said detectable group of agents in a sequence, wherein said sequence is comprising:

[a] applying to said immobilization support a blocking solution, thereby occupying all available sites on the support and,

[b] applying to said immobilization support a first detection agent which specifically recognizes the support-associated labeled ligand, and,

[c] removing of all the unreacted detection agent from the immobilization support and,

[d] applying to said immobilization support a subsequent detection agent which specifically recognizes the previous detection agent and,

[e] removing of all the unreacted subsequent detection agent from the immobilization support and,

[f] repeating [d] and [e] until all agents in the sequence are utilized;

wherein the final detection agent used in said sequence further comprises the detection agent which possesses a detectable moiety, and; wherein the number of said subsequent detection agents is unlimited, and; wherein if said number of subsequent agents is zero, said first detection agent comprises the entire sequence, thereby said first detection agent comprises the final detection agent, and thereby said first detection agent includes said detectable moiety, and; wherein determining the amount of immobilization support associated said final detection agent is performed by colorimetric, luminescent, chemical, or physical based detecting of said detectable moiety on said final detection agent, thereby producing said signal of claim 52.

Whereby increased sensitivity is afforded by the using of multiple subsequent detection agents.

Claim 68. (NEW) The method of claim 67, wherein said first detection agent includes an antibody to said labeled ligand.

Claim 69. (NEW) The method of claim 67, wherein said subsequent detection agent includes an antibody to the previous support-associated detection agent.

Claim 70. (NEW) The method of claim 67 wherein said blocking solution comprises a buffer solution containing a non-ionic detergent and a blocking reagent, wherein said blocking reagent is selected from a group consisting essentially of non-fat dry milk, and gelatin.

Claim 71. (NEW) The method of claim 67, wherein the said final agent detectable moiety includes conjugated enzymes.

Claim 72. (NEW) The method of claim 67, wherein said conjugated enzyme includes horseradish peroxidase.

Claim 73. (NEW) The method of claim 52, wherein exposing of said surface to the labeled ligand in varied conditions, whereby said insoluble surface is capable of deformation, is used to measure said surface labeled ligand binding or internalization.

Claim 74. (NEW) The method of claim 52, wherein the determining of the specific binding of the labeled ligand to said surface is performed by comparing the signals arising from the previously surface associated samples containing labeled and unlabeled ligand, to the signals arising from the previously surface associated samples containing labeled ligand only, thus ascertaining the competition for surface association between labeled and unlabeled ligand.

Add claims
10/002,690

In the Claims:

Please Cancel claims:

Claims 50,51 (canceled).

Text of all Claims:

Claims 1 – 35 (canceled)

Claim 36. (previously presented) A process for the evaluation of biological ligand binding and/or internalization using non-radioisotopic immunologically recognizable hapten-conjugated (labeled) ligands, consisting essentially of:

A. Applying a ligand possessing an immunologically recognizable hapten group (hereafter referred to as labeled ligand) to a surface; in the presence or absence of the same ligand which does not possess the immunologically recognizable group (hereafter referred to as unlabeled ligand).

B. Removing non-surface associated labeled and unlabeled ligand from the surface environment.

C. Solubilizing surface-associated labeled and unlabeled ligand; with or without disruption of the surface.

D. Optional: separating removed previously surface-associated labeled and unlabled ligand ; with or without concomitantly separating known amounts of labeled ligand.

E. Blotting of non-separated (C.) or separated (D.) solubilized previously surface associated labeled and unlabeled ligand onto a matrix; with or without concomitant blotting of known amounts of labeled ligand.

F. Detecting all blot matrix-associated, previously surface associated, labeled ligand, using a specific label recognizing entity; followed by specifically detecting that entity.

G. Optional: Determining the amount of blot matrix-associated previously surface-associated labeled ligand, by comparing its respective signals to the signals obtained from blot matrix-associated known amounts of labeled ligand.

Claim 37. (previously presented) The process as claimed in claim 36, wherein said surface is-comprised of, but is not limited to, biological cells.

Claim 38. (previously presented) The process as claimed in claim 36, where the immunologically recognizable group is: comprised of, but is not limited to, fluorescein, biotin, rhodamine, digoxygenin, or any other antibody-recognizable entity; Wherein said group is described as a hapten; Wherein said group is described as associated with the ligand by the term "conjugated".

Claim 39 (previously presented) The process as claimed in claim 36, wherein said biological ligand is comprised of, but is not limited to, transferrin, concanavalin A, avidin, annexin V, insulin, or any other protein, carbohydrate, nucleic acid, or any other substance or material which can possess said immunologically recognizable group.

Claim 40. (previously presented) The process as claimed in claim 36, wherein previously surface-associated labeled and unlabeled ligand can be separated by a method comprised of, but not limited to: electrophoresis, wherein such electrophoresis methodology consists of , but is not limited to sodium dodecyl sulfate polyacrylamide electrophoresis (SDS-PAGE), or other form of de-naturing or non-denaturing electrophoresis.

Claim 41. (previously presented) The process as claimed in claim 36, wherein said blotting method is comprised of, but is not limited to, dot blotting, slot blotting, or Western blotting.

Claim 42. (previously presented) The process as claimed in claim 36, wherein said blotting matrix is comprised of of, but is not limited to, cellulose, nitrocellulose, polyvinylidenediflouride (PVDF), or any other suitable blotting matrix.

Claim 43. (previously presented) The process as claimed in claim 36, wherein the detecting of blot matrix-associated labeled ligand is comprised of, but is not limited to, applying to the matrix an enzyme-conjugated or otherwise traceable anti-label antibody, followed by colorimetric, luminescent or other based detection of the antibody's enzyme or traceable entity.

Claim 44. (previously presented) The process as claimed in claim 36, where the detecting of blot matrix-associated labeled-ligand is comprised of, but is not limited to, application of an enzyme-conjugated or otherwise traceable avidin or streptavidin, followed by colorimetric, luminescent or other based detection of the avidin's or streptavidin's enzyme or traceable entity.

Claim 45. (previously presented) The process as claimed in claim 36, wherein the detecting of blot matrix-associated labeled material is comprised of, but is not limited to, applying to the matrix any sequence of antibodies; wherein the labeled ligand is detected, such as: applying an anti-label antibody, followed by applying an antibody to the anti-label antibody, followed by applying an antibody to the antibody to the anti-label antibody, etc.; with the final antibody possessing a conjugated enzyme or traceable entity. Wherein the amount of final antibody is determined by colorimetric, luminescent or other based detecting of the final antibody's enzyme or traceable entity.

Claim 46. (previously presented) The The process as claimed in claim 36, or 44, or 45, where the final antibody's traceable group is comprised of, but is not limited to, biotin; wherein the biotin is subsequently detected by applying to the blot matrix avidin or streptavidin possessing a conjugated enzyme or traceable entity . Wherein the amount of avidin or streptavidin is determined by colorimetric, luminescent or other based detecting of the avidin's or streptavidin's enzyme or traceable entity.

Claim 47. (previously presented) The process as claimed in claim 36, or 44, or 45, or 46, where the (final) antibody's, or avidin's, or streptavidin's conjugated enzyme is comprised of, but is not limited to, horseradish peroxidase, or alkaline phosphatase.

Claim 48. (previously presented) The process as claimed in claim 36, or 37, Wherein the exposure of said surface to the labeled ligand in varied conditions, followed by the same removing, separating, membrane binding, and detecting methods,

can be used to measure surface or cellular labeled ligand binding and/or internalization.

Claim 49. (previously presented) The process as claimed in claim 36, Wherein the specific binding of the labeled ligand to said surface can be determined by competitive binding with unlabeled ligand, followed by the same removing, separating, blotting, and detecting methods.

Claim 50 (canceled).

Claim 51 (canceled).